CHARACTERIZATION OF EXTRACTED FISH OIL FROM EEL: EFFECTS OF PROCESS PARAMETERS ON EXTRACTION YIELD. PARAMETERS:

c) DRYING TEMPERATURE
d) SOLVENT DIFFERENT

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DECLARATION

I declare that this thesis entitled "Characterization of Extracted Fish Oil from Eel: Effects of Process on Extraction Yield. Parameters: a) Drying Temperature, b) Solvent Different" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

Signature :……

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Date : 2 May 2009

DEDICATION

Special Dedication to my family members,
my friends, my fellow colleague
and all faculty members

For all your care, support and believe in me.

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ABSTRACT

Fish oil is recommended for a healthy diet because it contains the omega-3 fatty acids, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), that reduce inflammation throughout the body. The eel, scientifically known as *Monopterus albus* was used as the raw material. This study aimed at determining the best conditions to extract the fish oil in terms of drying temperature and solvents used for extraction process and to determine the compounds available in the extracted fish oil. The eel fillets were dried by using oven at five different temperatures ranging from 60 – 100 ^{o}C and then were grounded into powder form. The oil was extracted by using Soxhlet extractor for 6 hours and then was purified by using rotary evaporator to obtained concentrated oil. The fatty acid composition of the oil was analyzed using gas chromatography after being converted into methyl ester derivatives. Chemical quality of the oils which was acid value and free fatty acid composition were analyzed by using free fatty acid analyzer, 785 DMP Titrino. Results show that at temperature 70^{o}C with solvent ethanol were the best conditions to extract the fish oil. The acid values obtained are not beyond the accepted value thus, all the oils are in high quality. In the fatty acid analysis it was discovered that the major fatty acids in the oil were palmitic acid, stearic acid, oleic acid (omega-9) and linoleic acid (omega-6). As a conclusion, the best drying temperature and solvent to achieve high quantity of fish oil are 70^{o}C and ethanol respectively. The acid values reveal that the fish oils are in high quality. Four major fatty acid component detected in the fish oil are palmitic acid, stearic acid, oleic acid (omega-9) and linoleic acid (omega-6).

ABSTRAK

Minyak ikan telah disyorkan sebagai pemakanan yang sihat sebab ia mengandungi asid lemak omega-3, asid eikosopenaenoik (EPA), asid dokosahexaenoik (DHA), yang boleh mengurangkan keradangan. Belut, secara saintifiknya *Monopterus albus* (sejenis ikan air tawar) telah digunakan sebagai bahan ujikaji. Kajian ini bertujuan untuk menetukan keadaan yang paling baik untuk mengekstrak minyak ikan dari segi suhu pengeringan dan pelarut untuk proses pengekstrakkan dan juga menetukan komponen yang terdapat di dalam minyak belut. Isi belut dikeringkan menggunakan ketuhar pada 5 suhu yang berbeza dari suhu 60–100^{o}C dan kemudian dikisar menjadi serbuk. Minyak ikan diekstrak menggunakan pengekstrak Soxhlet selama 6 jam dan kemudian ditulenkan menggunakan penyejat berputar untuk mendapatkan minyak yang pekat. Komposisi asid lemak ditentukan menggunakan kromatografi gas setelah ditukarkan kepada terbitan metil ester. Kandungan asid dan kandungan asid lemak bebas ditentukan menggunakan penentu asid lemak bebas model 785 DMP Titrino. Keputusan menunjukkan pada suhu 70^{o}C dan pelarut etanol adalah keadaan yang paling baik untuk mengekstrak minyak ikan ini. Kandungan asid adalah tidak melebihi had yang diterima maka, semua minyak adalah berkualiti tinggi. Dalam analisis asid lemak, asid lemak utama di dalam minyak tersebut adalah asid palmitik, asid sterik, asid oleic (omega-9) dan asid linoleik (omega-9). Kesimpulannya, suhu pengeringan dan pelarut yang paling baik adalah 70^{o}C dan etanol. Minyak yang didapati adalah berkualiti tinggi. Empat komposisi asid lemak dalam minyak ikan ini ialah asid palmitik, asid sterik, asid oleik (omega-9) dan asid linoleik (omega-9).

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LIST OF SYMBOLS

g	-	gram
h	-	hour
mL	-	mililiter
%	-	percentage
°C	-	degree Celsius
µL	-	microliter
rpm	-	rotation per minute

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Asthma is characterized by overly reactive bronchi (increased "twitchiness"). This increased responsiveness, doctors and researchers believe, is due to underlying bronchial inflammation. The walls of the bronchi contain muscles, and the interiors are lined with a membrane (mucous membrane) that secretes mucus, or phlegm. In people with asthma, the bronchi decrease in size when they come in contact with certain triggering factors (Boutin, 1995). Doctors agree that the best treatment for asthma entails identifying and then avoiding its triggers. In some instances these are obvious for example exposure to tobacco smoke and other noxious fumes, cold air, exercise, or an allergy to animal dander. Seasonal asthma is usually due to various pollens, molds and other environmental factors (Schwarcz and Berkoff, 2004). Wheezing, chest tightness, labored breathing and other asthma symptoms occur when the tiny muscles that control the airways to the lungs constrict, causing a bronchospasm. Normally, the airways narrow somewhat when exposed to smoke, pollutants, very cold air or substances that are harmful if inhaled (Schwarcz and Berkoff, 2004). Heredity may be a factor too. The reason some people have hyperactive airways is unknown; heredity, however, suspected of playing a role, because the disease runs in the families (Schwarcz and Berkoff, 2004).

Most people with asthma cannot cure this disease but it can be controlled through preventive medicines and symptomatic treatments. Recently, fish and fish oil fatty acids are currently under intense scientific investigation because of the numerous

health benefits attributed to them (Rahman *et al*., 1995). Marine lipids are associated with high level of long chain omega-3 polyunsaturated fatty acid (PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Wu and Betchel, 2008). Many health experts suggest that two to three servings per week of seafood should be consumed in order to meet the recommended level of essential fatty acids for pregnant women, children and elderly people (Huhges, 1995; Olsen & Secher, 2002). Research studies done by expert found out that omega 3 fatty acids, particularly EPA, have a very positive effect on your inflammatory response. Through several mechanisms, they regulate your body's inflammation cycle, which prevents and relieves painful conditions like arthritis, prostatitis, cystitis and anything else ending in "itis" (Byrd, 2007)

In this study, swamp eel, *Monopterus albus* is used to extract the fish oil. Swamp eel naturally occurs in ponds, canals, ditches, rice fields, and swamps where it may be a dominant species (Smith, 1945). Asian swamp eels typically inhabit fresh-waters, but they may be found in brackish and saline waters (Nichols, 1943). It is no surprise that the eel's flesh is believed by many people to be the cure for kidney disease, asthma, heart palpitations and impotency as well as hastening healing of surgical wounds (www.bernama.com). In traditional practitioner, the eel soup is believed to cure asthma disease (www.yuhuii.com). Although there are not many studies on the eel benefits, it is verbally proven by the consumers of the meat where it can cure asthma disease. The reason to extract the swamp eel oil is because of its feature that looks like snake makes the consumers feel disgusted and afraid to consume the meat. The fish oil can be an alternative medicine to prevent asthma. Furthermore, this study is a preliminary research and later it will be a pioneer to do more research on this fish oil.

Extraction process is the best method to separate the oil from the fish. In this production of swamp eel oil, organic solvents such as ethanol, isopropanol and n-hexane are used to extract oil from the eel. Selection of extraction conditions depends on the nature of the extraction process, the temperature, pH and residence time could have an effect on the yield and selectivity (www.cheresource.com). Extraction process is conducted by using Soxhlet extractor. A Soxhlet extractor is a type of laboratory

glassware invented in 1879 by Franz von Soxhlet. This equipment is designed for the extraction of lipid from solid material, but also can use whenever it is difficult to extract any compound from the solid. The Soxhlet extractor enables solids to be extracted with fresh warm solvent that does not contain the extract. This can dramatically increase the extraction rate, as the sample is contacting fresh warm solvent. At the end of extraction, the excess solvent may be removed by using rotary evaporator, leaving behind the extracted lipid.

1.2 Problem Statements

A lot of studies had been conducted by using other types of fish species such as salmon and mackerel to produce fish oil. But studies on swamp eels are less known yet. This is due to lack of exposure on the benefits and also no proving conducted scientifically by the experts. Furthermore, there also no study conducted on the effects of process parameters on extraction yield where the parameters are on drying temperature an solvent used for extraction process.

Verbally mention by the consumers of the swamp eel said that by eating the fish meat it can reduce the risk of asthma disease. As mention above, some believed that by drinking the eel soup can also cure asthma disease. One of the problems of this study is where the swamp eel has a feature that looks like a snake that disgusted the consumers and some are afraid of it. In addition, swamp eel is difficult to catch due to its slippery body texture. Thus, this study is conducted to extract the swamp eel oil to give more pleasant consuming to the consumers. There are no researches documented scientifically of the contents inside the eel that can heal asthma disease. Doctors only prescribe medicines to keep asthma in control but there is no medicine produce for curing asthma disease. Furthermore, this is a preliminary research as there is not much evidence to prove the benefits of swamp eel oil. This study is conducted to search for the specific compound that gives benefits towards the asthma patients.

1.3 Objectives

The purposes of this study are to determine the best condition to extract fish oil in terms of drying temperature and solvent use for extraction process and to determine the compounds available in the extracted swamp eel oil.

1.4 Scopes of Study

Raw material used in this study is swamp eel. Preparation of the raw material before the process of extraction is needed to increase extraction yield. By knowing the objective of this study, drying temperature of the eel are varied in order to determine the best drying temperature to yield a lot of fish oil quantity. Drying is a process of removing water moisture by evaporation from a solid or semi-solid material. In this study, the fish is dried by using oven. Drying temperature ranges from 60^{o}C to 100^{o}C is used to dry the fish. Drying temperature of 60^{o}C is the lowest temperature use to dry the fish because of to evaporate the moisture content inside the fish very quickly. Hundred degree Celcius is the highest temperature choose to dry the fish because of to avoid any damages or any lost of biological components inside the fish where it is important to be determine during chemical analysis.

In the extraction process by using Soxhlet extractor, there are three solvents used to extract the fish oil which are ethanol, isopropanol and n-hexane. These three solvents are choose because it is easy to get from any chemical supply companies in Malaysia.

The compounds inside the fish oil is identify by using Gas chromatography and also the acid value is quantified to measure the quality of the fish oil.

1.5 Rationale and Significance

The rationales of doing this study are:

1. To increase the extraction yield by monitoring parameters so that the pure oil can be used effectively.
2. To do preliminary research on the extracted oil using swamp eel. This study is a pioneer toward further research and studies.
3. To do clinical test on the swamp eel oil for determination of certain compounds that gives cure to asthma disease.
4. To open up opportunities to increase the demand on manufacturing the swamp eel oil in the form of capsules as proven by the researchers that fish oil have a lot of benefits.
5. To enhance the medication treatment for asthma illness and to research for alternative medicines that give benefits to the asthma patients.

CHAPTER 2

LITERATURE REVIEW

2.1 Eel

Eel, scientifically known as *Monopterus albus*, naturally occurs in ponds, canals, ditches, rice fields, and swamps where it may be a dominant species (Smith, 1945). Asian swamp eels typically inhabit fresh-waters, but they may be found in brackish and saline waters (Nichols, 1943).

The body is more or less cylindrical; tail compressed tapering to a slender point much shorter than the trunk. Scales are absent. The snout is bluntly rounded, the jaws and palate have rows of viliform teeth. The upper lip is thick overlapping part of the lower lip. The eye is small, covered by a layer of skin (Nichols, 1943; Jayaram, 1981). Body color is slate brown above, white or light-brown below with small dark spots on sides and occasionally on the ventral surface (Inger and Kong, 1962). The lateral line is well developed and conspicuous (Jayaram, 1981). In adults, paired fins are lacking, and the dorsal, caudal and anal fins are reduced (Nichols, 1943; Sterba, 1983). Although specimens over 70 cm are relatively rare, this species may grow to a meter in length. Most grow to between 25 and 40 cm (Smith, 1945).

Figure 2.1: Eel (www.wikipedia.com)

This species occur in Asian country such as India, China, Japan, Malaysia and Indonesia. Probably also occurring in Bangladesh (www.fishbase.org). Based on the Annual Fisheries Statistic 2004 (Volume 1) by Department Of Fisheries, Malaysia, estimated fish production from public waterbodies (rivers) shows that only in Terengganu (0.2 tonnes) and Kelantan (2.80 tonnes) is the main producer of swamp eel. The estimated wholesale and retail value are as in Table 2.1

Table 2.1: Estimated wholesale and retail value of swamp eel in 2004, RM '000 (Annual Fisheries Statistic 2004, Department of Fisheries)

State	Wholesale value	Retail value
Terengganu	1.10	1.60
Kelantan	13.92	20.02

Even though many people are quite reluctant to eat the eel, it cannot be denied that this freshwater fish has many nutritional benefits. Its nutritional values are said to be on par with that of the 'tenggiri' (Spanish mackerel) and 'selar' (crevelle) which have 18.6 per cent protein and 15 per cent fat. The eel is also rich in calcium and iron as well as vitamins B and D. (www.bernama.com)

According to Razak *et al,* the eel, *Monopterus albus* contain a high level of docosahexanoic acid (DHA) which is 6.21 g/100 g lipid. Study by Rahman et al shows amount of eicosapentanoic acid (EPA) were found to be less than 3.5 % with freshwater eel being the highest (3.48 %) followed by eel (2.66 %). This would explain the usage of these two fish as a traditional medicine for muscles pain (Mohsin & Ambak, 1983).

2.2 Fish Oil

The fatty acid composition of marine lipids varies significantly, especially when compared with vegetable oils (Shahidi, 2005). Fatty acids can be divided into two which are saturated fatty acids or unsaturated fatty acids. Fish oils are rich in monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), and they are good sources of omega-3 PUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Shahidi, 2005). Omega-3 PUFA has benefits towards human health. These fatty acid decrease the stickiness of blood platelets, making it less likely that they will clump together to form clots. They also increase the flexibility of red blood cells, enabling them to pass more readily through tiny vessels, reduce inflammation of the artery walls and lower levels of triglycerides in the blood (Schwarcz and Berkoff, 2004). The human body uses omega-3 fatty acids to manufacture prostaglandins, chemicals that play a role in many processes, including inflammation and other functions of the immune system (Schwarcz and Berkoff, 2004). Not only the human need PUFAs to give them a lot of benefits, but fish also need it for their own good. Fish need PUFAs to provide tolerance to low water temperatures (Rahman *et al.,* 1994).

The fat content in fish varies according to seasons, species and geographical variation. Age variation and sex maturity in the same species also contribute to the significant differences in the total lipid contents (Piggott & Tucker, 1990; Tsuchiya, 1961; Rahman *et al.,* 1994). Researchers have found that freshwater fish contain lower proportions of omega-3 PUFA than do marine fish (Rahman *et al*., 1994). Decreases in PUFA concentrations in lipids would therefore be expected in warmer

waters (nearer the equator) like Malaysia. Wang et al. (1990) found that the ω-3: ω-6 ratio of freshwater fish was lower than marine fish. Freshwater fish normally consist of more omega-6 PUFA whereas the marine fish are rich in omega-3, especially DHA and EPA (Wang *et al.,* 1990). Figure below shows the chemical structure of DHA and EPA.

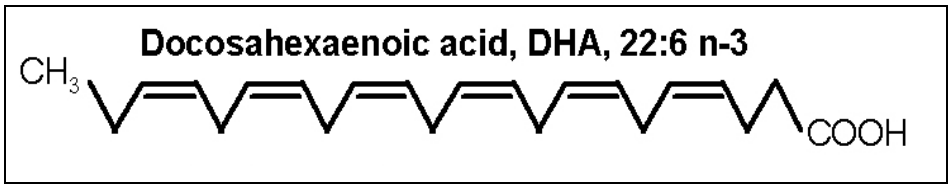

Figure 2.2: Chemical structure of docosahexaenoic acid (DHA)

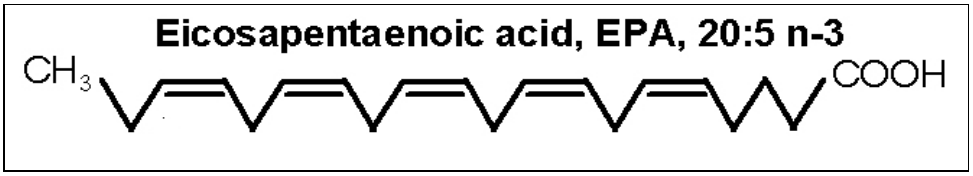

Figure 2.3: Chemical structure of eicosapentaenoic acid (EPA)
(www.omega-3-forum.com)

2.3 Asthma

Currently available therapy for asthma is highly effective and, if used appropriately, usually has no problems in terms of adverse effects. However, some patients (5–10% of asthmatic patients) remain poorly controlled, despite what appears to be optimal therapy (Barnes *et al*., 1998). In this study, the fish oil is used as an alternative medicine for curing or preventing asthma disease.

2.3.1 Asthma Disease

Asthma is characterized by overly reactive bronchi (increased "twitchiness"). This increased responsiveness, doctors and researchers believe, is due to underlying bronchial inflammation. The walls of the bronchi contain muscles, and the interiors are lined with a membrane (mucous membrane) that secretes mucus, or phlegm. In people with asthma, the bronchi decrease in size when they come in contact with certain triggering factors. The triggering factors can be due to exposure to smoke, pollutants, very cold air or substances that are harmful if inhaled (Schwarcz and Berkoff, 2004). However, the response in exaggerated and often triggered by otherwise harmless substances or activities, such as pollen and other allergens and exercise (Schwarcz and Berkoff, 2004).

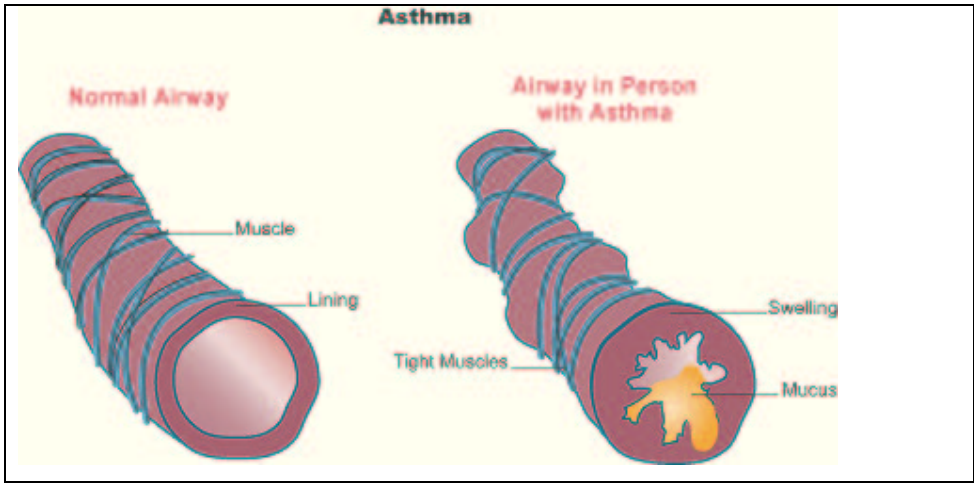

Figure 2.4: Lung airway in normal person and asthma patient (http://www.nhlbi.nih.gov)

The bronchi in young children, which are smaller, are more easily obstructed. During an asthma attack, the following changes take place in the bronchi and bronchioles:

1. The muscles encircling the bronchi contract, their interior diameter (lumen) narrows and air cannot reach the lungs as easily (in medical terms, this phenomenon is called bronchospasm).

2. The membrane lining the inside of the bronchi becomes inflamed and swollen, making it even more difficult for air to pass through (this is inflammation).

3. Excess secretions can lead to the formation of mucus plugs, which reduce the air passages even more (Boutin, 1995).

There are no specific foods that prevent asthma, but some may lessen its complications. Omega-3 fatty acids, found in salmon, mackerel, sardines and other cold-water fish, have an anti-inflammatory effect and may counter bronchial inflammation (Schwarcz and Berkoff, 2004).

2.3.2 Medication

Once asthma has been diagnosed, the goal of treatment is to minimize or eliminate respiratory symptoms and restore normal lung function by:

- Identifying the triggering factors and effectively correcting the environment.
- Using the minimum of drugs appropriate to the severity of the asthma;
- Preventing and treating flare-ups early and effectively (using a written action plan).

Medication varies according to the severity of asthma in each person. That person must know and understand:

- How to use asthma drugs and what effects they have
- What drugs you must take

- How to change medication if asthma symptoms worsen;
- When to consult a doctor.

Today, people take asthma medication mainly by inhalation. An inhaled drug can be taken at a lower dose and thus causes fewer side-effects than if it was taken in the form of pills or syrup (Boutin, 1995). In this study, the fish oil is extracted from flying fish as an alternative medicine to prevent asthma disease. This alternative medicine is basically free from chemicals. Chemicals base medicines are not very good in long term because it might cause harm to human body if it is not suitable to them.

2.4 Factors effecting extraction process

There are many factors that can affect extraction process. In this study focuses more on drying temperature and solvent different.

2.4.1 Drying

Drying generally means removal of small amount of water from material. In drying, the water is usually removed by as vapor in the air (Geankoplis, 2003). The moisture content of the final dried product varies for different material to dry. Dried salt contains about 0.5% water, coal about 4% and many foods products about 5 % (Geankoplis, 2003). Several different ways and methods can be classified for drying processes. Drying processes can be done in batch, where the material is inserted in the drying equipment and drying process proceeds for given period of time. Drying also can be done in continuous where the material is continuously added to the dryer and dried material is continuously removed. The drying temperature range of 60^{o}C to 100^{o}C is used to dry the filleted fish. Predrying facilitates the grinding of the samples, enhances extraction and may break fat-water emulsions to make fat dissolve easily in the organic solvent and helps to free tissue lipids (Akoh and Min, 2002). Drying the